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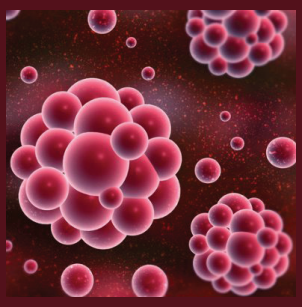

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Research Article: Carbapenemase Producing *Klebsiella* species from Hospitalised Patients at Three Referral Hospitals in Yaounde; Cameroon

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Abstract:

Background: Carbapenemases are the most potent beta-lactamases capable of hydrolysing all beta-lactam drugs. Data scarcity on laboratory evidence of carbapenemase producers obstructs infection prevention and effective control measures. This study aimed to detect and phenotypically characterize *Klebsiella* species resistant to carbapenems.

Method: A total of eighty-seven (87) *Klebsiella* spp. isolates were collected from three Yaounde-based tertiary hospitals over a period of twelve months (February 2019 to January 2020). The isolates were identified on API 20E and antimicrobial susceptibility testing was done according to the Kirby Bauer disc diffusion method. Carbapenem resistance was detected based on reduction in inhibition zone diameter of meropenem less than 28mm and resistance phenotypes determined by the combination disc synergy test.

Results: Four *Klebsiella* species were identified namely: *Klebsiella pneumoniae* 62/87 (71.3%), *K. oxytoca* 11/87 (12.6%), *K. ozaenae* 7/87 (8%), *K. rhinoscleromatis* 7/87 (8%). *Klebsiella pneumoniae* was most resistant to carbapenems with a considerably high number of multidrug resistant isolates 39/56 (69.6%). Patients from the 0-9 years age group were most represented 29/87 (33.3%). Carbapenemases belonging to Ambler classes A, B and D were detected as follows: KPC 2/87 (2.3%), MBL 2/87(2.3%), OXA-48 4/87 (4.6%). All four *Klebsiella* species were producers of KPC carbapenemase 12/87 (13.8%) and Extended Spectrum β-lactamase 68/87 (78.2%) enzymes.

Conclusion: These results revealed that more than half of *Klebsiella* species analysed were multidrug resistant and about a quarter in hospitalized patients at the referral hospitals were mostly KPC carbapenemase producers. This raises concerns over the need to control and confine the spread of these isolates of clinical and epidemiological importance for effective infection prevention and control.

Keywords: Carbapenems, Carbapenemase, Resistance, *Klebsiella pneumoniae* carbapenemase, Metallo beta-lactamase, OXA-48

Introduction

Background: Carbapenems are a class of beta-lactam antibiotics with very broad activity against extended spectrum beta lactamase (ESBL) producing bacteria and multidrug resistant Gram-negative bacteria. These multidrug resistant bacteria in addition to resistance determinants against β-lactam drugs usually harbour a combination of non-β-lactam resistance genes against fluoroquinolones and aminoglycosides (1). Resistance to carbapenems is either due to an acquired carbapenemase(s) that enzymatically breaks down carbapenems or the production of an ESBL/Class C Cephalosporinase (AmpC) combined with porin loss (1) (2). Carbapenemase producers pose a big clinical threat by compromising the antimicrobial activity of last resort antibiotics used in hospitals for the treatment of serious infections caused by multidrug-resistant bacteria. In immunocompromised patients and high-risk patients such as hospitalized patients at intensive care units, rectal colonization with Carbapenemase Producing Enterobacteriaceae (CPE) and prior antibiotic use are risk factors for subsequent endogenous CPE infections and cross-transmission among patients (3).

Dissemination of carbapenemase producers is both an epidemiological and clinical problem because resistance genes found on self-conjugative plasmids carrying other resistance determinants, easily spread vertically and via horizontal gene transfer to related Gram-negative bacilli (4) (5) (6) causing cross-resistance. This transforms otherwise antibiotic susceptible strains into non-susceptible strains. (7).

Enterobacteriaceae are Gram-negative bacteria commonly encountered in clinical samples and they may be responsible for approximately 50% of all clinically significant bacteria (8). The emergence of carbapenemase resistant Enterobacteriaceae (CRE) over the past decades in many parts of the world including Japan, United Kingdom, Greece, United States of America, Italy, India and some Central and South American countries, usually involves *Klebsiella pneumoniae and Escherichia coli* (9) (10) (11). Rapid clonal dissemination of acquired carbapenemases as a result of class A (KPC), class B metallo-β lactamases (IMP, VIM, NDM) or class D (OXA-48) serine carbapenemases complicates patient outcome. Sizing up the problem of CPE in Africa and precisely Cameroon is difficult because of the absence of survey reports, published studies, absence of effective infection prevention and control practice in healthcare settings, gaps in training for the detection of CPE and poor diagnostic tools (12) (13). In this context when therapeutic failure occurs, given the limited diagnostic and treatment options, dissemination of undiagnosed and untreated CPE continues to be promoted. This study aimed to detect and phenotypically characterize *Klebsiella* species resistant to carbapenems and to contribute to antimicrobial resistance stewardship.

Method and materials

Study design and study population

This study was cross-sectional and descriptive carried out over a period of one year (February 2019 to January 2020). The study population was the portion of hospitalized patients of all ages who gave non-repetitive clinical specimens for analysis at the hospital and therein *Klebsiella spp.* was isolated. Eighty-seven (87) *Klebsiella spp.* isolates were obtained.

Description of study site

The three referral hospitals namely: the Yaounde University Teaching Hospital (YUTH), the Yaounde General Hospital (YGH) and the Yaounde Gynaeco-Obstetrics and Paediatrics Hospital (YGOPH) situated within the urban setting of Yaounde and offering medical services of diverse speciality to both in and out-patients. The consulted patient population is cosmopolitan in terms of ethnicity, age, and sex. Microbiology analysis was carried out both at the hospitals and at the Centre for the Study and Control of Communicable Diseases, Faculty of Medicine and Biomedical Sciences, The University of Yaounde I.

Ethical clearance

This research work was approved by the *Comité Institutionnel d'Ethique de la Recherche pour la Santé Humaine, Université Catholique d'Afrique Centrale, Ecole des Sciences de la Santé* registration number No.2019/020178/CEIRSH/MI.

Data collection

A Structured questionnaire was administered to all consenting participants and clinical information was obtained from hospital registers with the assistance of laboratory and ward staff.

Isolation of *Klebsiella* spp.

Clinical specimens were cultured on eosin methylene blue agar (EMB). Based on colony morphology, suspected 3-5mm lactose positive and mucoid colonies were purified on nutrient agar. Biochemical identification of suspected *Klebsiella* spp. was carried out on API 20E testing system (Biomerieux, Marcy-l'Etoile, France) according to the procedure referenced by the manufacturer (14).

Antimicrobial susceptibility testing

Twenty eight antibiotics (from Rapid Labs Ltd, Colchester ESSEX-UK) belonging to four families (β-lactams, aminoglycosides, sulphonamide and quinolones) were tested according to the Kirby Bauer disc diffusion method (15). *Escherichia coli* ATCC 25922 was used for quality control of the antibiotic discs.

Determination of resistance

Screening for ESBL, AmpC and carbapenemase producers was simultaneously done with antimicrobial susceptibility testing of third generation cephalosporins, cefoxitin and carbapenems respectively. Each suspected ESBL producing isolate, was confirmed based on the combination disc testing method. Discs containing cefotaxime and cefotaxime clavulanate were applied on an inoculated Mueller Hinton plate. After 18 to 24 hours aerobic incubation at 35+/- 2°C, the inhibition zones around the cefotaxime disc and cefotaxime clavulanate were compared. The isolate was considered an ESBL producer if the inhibition zone diameter was equal to or greater than 5 mm around the cefotaxime clavulanate disc compared with cefotaxime disc. This phenotype is illustrated in figure 1. *Escherichia. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 strain were used for quality control.

The detection of a plasmid-mediated AmpC-type cephalosporinase producer was based on cefoxitin zone inhibition diameter less than 19 mm, an inhibition zone diameter equal to or greater than 5 mm around the cefoxitin clavulanate disc compared with cefoxitin disc, alongside phenotypic resistance to ceftazidime and or cefotaxime (fig 1).

Carbapenemase screening was based on the reduction in diameter of inhibition around meropenem discs. The screening breakpoint of meropenem considered was an inhibition zone diameter greater than or equal to 28mm. (16). Confirmation of a carbapenemase producing isolate was based on the combination disk synergy testing method. Synergy between a meropenem disc and meropenem in combination with various inhibitors (boronic acid, EDTA, cloxacillin) and reduction of the inhibition zone diameter around temocillin disc in the absence of synergy confirmed a carbapenemase producer as illustrated in figures 2-4. All the confirmation antibiotic discs used were produced by Liofilchem, Roseto degli Abruzzi-Italy.

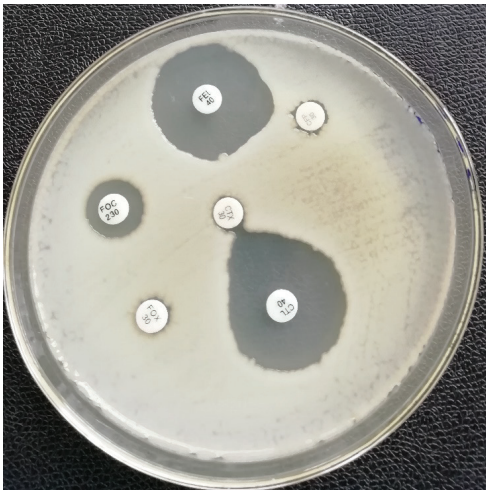

Figure 1: ESBL and AmpC producer [centre CTX-cefotaxime disc, clockwise CFP-cefepime disc, CTL-cefotaxime clavulanate disc, FOX-cefoxitin disc, FOC-cefoxitin clavulanate disc, FEL-cefepime clavulanate disc] (Source of image: present study)

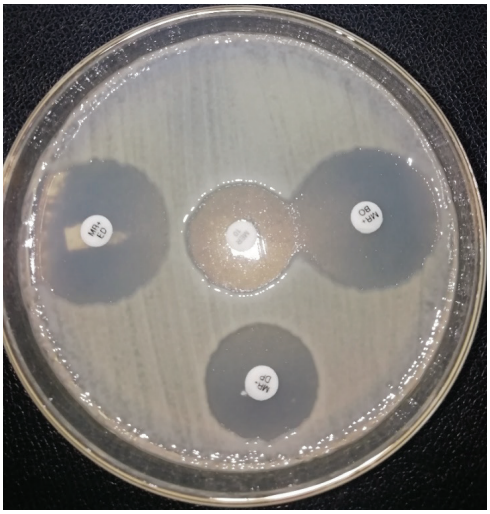

Figure 2: *Klebsiella pneumoniae* carbapenemase (KPC) [centre MER-meropenem disc, clockwise MR+BO-meropenem + boronic acid disc, MR+CL-meropenem + cloxacillin disc, MR+ED- meropenem+EDTA disc] (Source of image: present study)

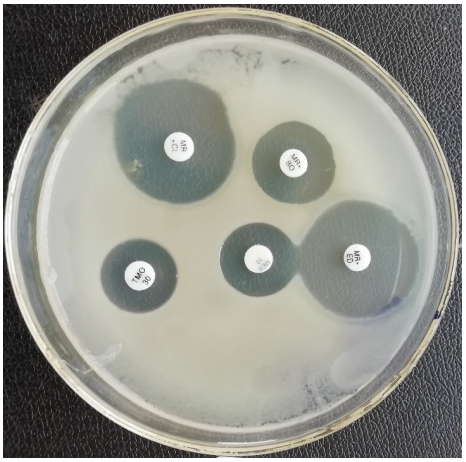

Figure 3: Metallo-beta-lactamase (MBL) carbapenemase [centre MER-meropenem disc, clockwise MR+BO-meropenem + boronic acid disc, MR+ED- meropenem+EDTA disc, TMO-temocillin disc, MR+CL-meropenem + cloxacillin disc] (Source of image: present study)

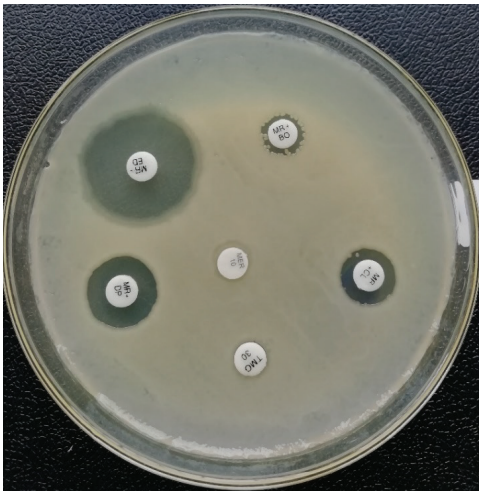

Figure 4: OXA-48 carbapenemase [centre MER-meropenem disc, clockwise MR+BO-meropenem + boronic acid disc, MR+CL-meropenem + cloxacillin disc, TMO-temocillin disc, MR+ED- meropenem+EDTA disc] (Source of image: present study)

Statistical analysis

Statistical analysis was done with the aid of CS Pro 7.1 and MS Excel softwares. A p-value less than 0.05 was considered statistically significant.

Results

Eighty-seven [87] *Klebsiella* spp. isolates were obtained distributed as follows:

K. pneumoniae 62/87 (71.3%), *K. oxytoca* 11/87(12.6%), *K. ozaenae* 7/87(8%) and *K. rhinoscleromatis* 7/87 (8%).

Participants were recruited from all wards and age groups ranging from one day old to eighty-seven years old, with a mean age of 31 years. Most participants belonged to the age group 0-9 years with a frequency of 29/87 (33.3%) while the 30-39 years age group was least represented with a frequency of 3/87 (3.4%). The majority of participants were females accounting for a frequency of 50/87 (57.5%) as opposed to males 37/87 (42.5%). Thirty-six [36] isolates were collected from the Yaounde University Teaching Hospital, seventeen [17] from the Yaounde General Hospital and thirty-four [34] from the Yaounde Gynaeco-Obstetrics and Paediatrics Hospital. *Klebsiella* spp. were isolated from eleven clinical specimens, mostly from urine 32/87. Within the 0-9 years age group, most isolates were recovered from urine 15/29 (51.7%) and blood 13/29 (44.8%).

The resistance rates of isolates to the various classes of antibiotics were concordant with current high resistance trends to third generation cephalosporins (70.9%), fourth generation cephalosporins (77.7%), aminoglycosides (44%), sulphonamide (94 .5%) and fluoroquinolones (39.8%) and low resistance rates to carbapenems (7.5%). Several isolates 56/87 (64.4%) were multidrug resistant and as expected the bulk of them was *K. pneumoniae* 39/56 (69.6%). Although overall, *K. oxytoca* isolates were fewer than *K. pneumoniae* isolates, at intraspecies level, *K. oxytoca* recorded more multidrug resistant isolates 8/11 (72.7%). Carbapenemases belonging to all three Ambler classes were detected either as single β-lactamase or in combination with ESBL or AmpC. Class A carbapenemase KPC represented 2/87 (2.3%), class B MBL 2/87(2.3%), and class D OXA-48, 4 /87 (4.6%) enzymes were detected in some isolates. All four *Klebsiella* species were producers of KPC carbapenemases 12/87

(13.8%) and Extended Spectrum β-lactamases 68/87 (78.2%). One isolate that was resistant to imipenem and sensitive to meropenem was expressed as an ESBL+ porin loss phenotype. There were two isolates that were intermediate to meropenem and susceptible to imipenem. One expressed ESBL+ porin loss and the other ESBL+ AmpC phenotypes. (Table 1)

Table 1: Results of antimicrobial profiles and resistance phenotypes

Parameters	*K. pneumoniae*	*K. oxytoca*	*K. ozaenae*	*K. rhinosclero-matis*	Total
Bacteria count	62 (71.3%)	11 (12.6%)	7 (8.0%)	7 (8.0%)	87 (100.0%)
Isolate count in most represented age group	22	2	2	3	29
Isolate count YUTH	23	4	4	6	37
Isolate count YGH	11	4	1	0	16
Isolate count YGOPH	29	3	2	1	35
Multidrug resistant isolates	39 (62.9%)	8 (72.7%)	5 (71.4%)	4 (57.1%)	56 (64.4%)
Resistance to penicilins	97.6%	92.9%	96.3%	100%	96.7%
Resistance to third generation cephalosporins	66.6%	61.9%	74.3%	80.9%	70.9%
Resistance to fourth generation cephalosporins	63.6%	71.4%	75.8%	100%	77.7%
Resistance to monobactam	63.6%	71.4%	72.6%	85.7%	73.3%
Resistance to carbapenems	4.6%	0%	3.2%	0%	2.0%
Resistance to sulphonamide	90.9%	100%	87.1%	100%	94.5%
Resistance to aminoglycosides	24.6%	53.6%	40.7%	57.15%	44%
Resistance to quinolones	37.9%	42.9%	45.2%	33.4%	39.84%
ESBL phenotype	63,6%	71,4%	66,1%	100,0%	74.3%
ESBL, AmpC phenotype	9,1%	0,0%	8,1%	0,0%	4.3%
ESBL, porin loss phenotype	0,0%	0,0%	3,2%	0,0%	0.8%
None ESBL phenotype	27,3%	28,6%	22,6%	0,0%	14.6%
AmpC phenotype	9,1%	0,0%	0,0%	0,0%	2.3%
AmpC, ESBL phenotype	9,1%	0,0%	8,1%	0,0%	4.3%
None AmpC phenotype	81,8%	100,0%	90,3%	100,0%	93.0%
KPC phenotype	9,1%	14,3%	0,0%	0,0%	5.9%
MBL phenotype	0,0%	0,0%	3,2%	0,0%	0.8%
OXA-48 phenotype	9,1%	0,0%	4,8%	0,0%	4.5%
None carbapenemase phenotype	81,8%	85,7%	75,8%	85,7%	82.3%

Discussion

Our study sought to determine the antimicrobial resistance profile and carbapenemase phenotypes of *Klebsiella* spp. isolated from hospitalized patients at three referral hospitals in Yaounde. Isolates were recovered most from participants within the age group 0-9 years and from two clinical specimens namely urine 15/29 (51.7%) and blood 13/29 (44.8%). This indicates that hospitalized children within this age group from whom *Klebsiella* spp. were isolated suffered most from urinary tract infections (UTIs) and bloodstream infections. Furthermore, among the 29 isolates recovered from this age group, only 4/29 (13.7%) were none-β-lactamase producers while 25/29 isolates produced either ESBL alone 16/29 (55.2%) or in combination with other β-lactamases such as: carbapenemases (7/29) and AmpC (3/29). The consequences for these vulnerable patients with immature immunity can be treatment failure and even death as a result of sepsis or in the case of UTIs, the infection may become chronic and result in kidney scaring, hypertension and renal failure (17).

Multidrug resistant *Klebsiella* spp. have been prominently described (6), (18), (19), (20) , (21) (22). Multidrug resistant isolates concomitantly carry resistant genes to several classes of antimicrobials like β-lactams, aminoglycosides and fluoroquinolones. Most of our isolates 56/87 (64.4%) were multidrug resistant and more than half were *K. pneumoniae* (39/56, (69.6%), the species with wide-ranging ecological distribution, high antimicrobial resistance gene diversity, plasticity and a higher plasmid burden (23). The interplay of the genetic disposition of *K. pneumoniae* makes it better organized for mobilizing resistant genes from other drug resistant bacteria in the environment or in animal/human microbial communities. This further explains why *K. pneumoniae* is always the predominant species as was the case in an earlier study (19). Such information is fundamental for shaping effective routine analysis of clinical specimens.

Resistance to carbapenems is a serious problem because they consist of one of the last treatment options for serious infectious diseases and the genes responsible for their resistance are transferable among Enterobacteriaceae. Resistance to carbapenems is either due to an acquired carbapenemase(s) which will enzymatically break down carbapenems or as a result of the production of an ESBL/Class C Cephalosporinase (AmpC)

combined with porin loss (1) (2). Carbapenemases are the most clinically important mechanism of carbapenem resistance and their global trend is on the rise (4), (6) (22), (24). In this study, carbapenemases belonging to all three Ambler classes were detected and most of them were detected in combination with other β-lactamases like ESBL or AmpC. ESBLs render isolates resistant to third, fourth generation cephalosporins and monobactams, and AmpC confer resistance to third generation cephalosporins and cephamycins (16). Three isolates that were intermediate or resistant to either imipenem and meropenem did not produce carbapenemases but rather ESBL + porin loss and/or AmpC which are known to induce low-level resistance to carbapenems (16). This is an indication of diversity in resistance clones within our research setting especially as all four *Klebsiella* species were producers of KPC.

Class A and B carbapenemases: KPC 2/87 (2.3%), MBL 2/87(2.3%) respectively, KPC and MBL 11/87 (12.6%) were most detected compared to Class D OXA-48 type enzymes (27) representing 4/87 (4.6%) of carbapenemases. This tendency reflects the situation in parts of Africa where the molecular epidemiology of carbapenemase genes have been described (North Africa, South Africa, West and East African countries), predominantly carbapenemase genes of *bla*OXA-48 type, *bla*IMP, *bla*VIM, and *bla*NDM in *Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae, Citrobacter spp., and Escherichia coli* (25), (26). Although risk factor assessment of CPE patient-to-patient transmission was not carried, it has been established that, transmission is common in patients experiencing long-term hospitalisation and without prior screening for CPE colonisation at the time of admission (12). In resource limited countries such as ours, absence of screening for CPE colonisation at patient admission is a setback to efforts at CPE prevention and control.

Conclusion

Evidence from this study on carbapenemase resistance phenotypes and various combinations of co-expressed mechanisms reveal that *K. pneumoniae* and to an extend *K. oxytoca* isolates were quite assorted. More than half of *Klebsiella* species analysed were multidrug resistant and about a quarter of *Klebsiella* spp. isolates in circulation in referral hospitals in Yaounde were carbapenemase producers. This raises concerns over the need to control and confine the spread of these isolates of clinical and epidemiological importance for antimicrobial stewardship as well as for effective infection prevention and control.

List of abbreviations

AmpC- ampicillinase cephalosporinase
blaIMP- beta-lactamase imipenem
blaNDM- beta-lactamase New Delhi metallo-β-lactamase
blaOXA-48 - beta-lactamase oxacillanase-48 type
blaVIM, - beta-lactamase Verona integron-encoded metallo-β-lactamase
CPE – carbapenem producing Enterobacteriaceae
EDTA- ethylenediaminetetraacetate
ESBL- Extended spectrum beta-lactamase
KPC- *Klebsiella pneumoniae* carbapenemase
MBL- metallo-β-lactamase
PBA- Phenyl Boronic acid
UTIs- urinary tract infections
YGH - Yaounde General Hospital
YGOPH- Yaounde Gynaeco-Obstetrics and Paediatrics Hospital
YUTH- Yaounde University Teaching Hospital

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